

United States Department of Agriculture  
Grain Inspection, Packers and Stockyards Administration  
Federal Grain Inspection Service

# FGIS Issuance Change

CHANGE TO			
9 DIRECTIVE		9 MANUAL	X9 HANDBOOK
CHANGE NO: 7	TO (No.)	TITLE: Aflatoxin Handbook	DATE: 3-17-03

**PURPOSE OF CHANGE:** The Aflatoxin Handbook has been revised to remove the EZ-Screen test kit as an FGIS-approved test method, condense sample preparation procedures, eliminate the use of "estimated" test results, and provide specific information on test equipment (i.e., readers) requirements.

## FILING INSTRUCTIONS

Remove	Dated	Insert	Dated
Table of Contents	6-24-02	Table of Contents	3-17-03
Pages 1-3, 1-4	6-24-02	Pages 1-3, 1-4	3-17-03
Chapter 3	3-4-02	Chapter 3	3-17-03
Chapter 4	3-4-02	Chapter 4	3-17-03
Chapter 6	3-4-02	Chapter 6 (reserved)	no date
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Retain this issuance sheet as an aid in verifying handbook contents.

*/s/ David Orr*

David Orr, Director  
Field Management Division



U.S. DEPARTMENT OF AGRICULTURE  
GRAIN INSPECTION, PACKERS AND STOCKYARDS  
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AFLATOXIN HANDBOOK  
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Aflatoxin Testing

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## 1.5 APPROVED TEST METHODS

FGIS has approved test kits for use at field testing locations. The AflaCup, EZ-Screen, and Agri-Screen test kits are approved for qualitative analysis of corn. The Aflatest, Fluoroquant, Veratox-AST, and Myco✓ test kits provide quantitative analysis but can be used for qualitative results. High Performance Liquid Chromatography (HPLC) testing is reserved for quantitative testing at the Technical Services Division (TSD) only.

The methods listed below have been conformance tested to perform within FGIS specifications. Each of the approved test methods has been certified to provide results accurate up to the conformance test level at which they were approved.

FGIS APPROVED TEST METHODS			
Method and Test Kit	Approved for		Conformance Limit(s)
	Qualitative	Quantitative	
AflaCup (International Diagnostics Inc.)	X		20 ppb
AgriScreen - (Neogen)	X		20 ppb
Veratox AST - (Neogen)	X	X	300 ppb (quantitative)
Fluoroquant - (Romer)	X	X	300 ppb (quantitative)
Aflatest - (Vicam)	X	X	300 ppb (quantitative)
Myco✓ - (Strategic Diagnostics Inc.)	X	X	300 ppb (quantitative)

Listed in the table below are the test kits that are commonly used for official aflatoxin analysis. Use the table to determine the appropriate test kit(s) to use for testing the listed grain/commodity. For information concerning the testing of mixed grain, contact the Policies and Procedures Branch.

GRAIN/ COMMODITY	TEST METHOD					
	AflaCup	Aflatest	Agri-Screen	Fluoroquant	Veratox-AST	Myco✓
Corn	X	X	X	X	X	X
Sorghum		X		X	X	X
Wheat		X		X	X	
Soybeans		X		X	X	
Corn Screenings		(*)			(*)	
Corn Meal		X		X	X	X
Corn Germ Meal		X			X	
Corn Gluten Meal		X			X	
Corn/Soy Blend		X		X	X	X
Corn Gluten Feed		X				
Flaking Corn Grits		X		(*)	(*)	
Corn Flour					(*)	
Corn Bran					(*)	
Popcorn		X		X	X	X
Milled Rice		X		X	X	
Rough Rice					(*)	
Cracked Corn	(*)	(*)	(*)	(*)	(*)	(*)

**NOTE:** An X entered into a block denotes that the test kit has been evaluated and approved for the grain/commodity.

The symbol (\*) entered into a block denotes that the test kit is under evaluation by TSD Division for the grain/commodity and is temporarily approved for official use.

## CHAPTER 3

### SAMPLE PREPARATION

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### 3.1 GENERAL INFORMATION

The manner in which samples are obtained and processed is an important consideration when testing for aflatoxin. To ensure that the test results accurately reflect the aflatoxin concentration present in a lot, samples must be representative of the lot and of sufficient size to compensate for the uneven distribution of the contaminant.

### 3.2 SAMPLE SIZE

Obtain samples according to the instruction in the Grain Inspection Handbook, Book I, "Grain Sampling."

The minimum sample size is based on the type of lot. Applicants may request a sample size larger than the minimum sample size.

Lot Type	Minimum Sample Size (lbs.)/ grams
Trucks	2 pounds / approximately 908 grams
Railcars	3 pounds / approximately 1,362 grams
Barges/Sublots	10 pounds / approximately 4,540 grams

**NOTE: A minimum sample size of 10 pounds is required for composite type samples (e.g., a single sample representing multiple carriers). A 10-pound sample size is also recommended, but not required, for submitted samples.**

### 3.3 WORK RECORDS

Each testing laboratory must maintain work records for each test that include the name of the applicant, date of service, sample or carrier identification, test results, initials of official personnel performing the test, and any other information deemed necessary to properly certificate the test results and bill the applicant. As practical, use existing forms, such as FGIS-992, "Services Performed Report;" FGIS-920, "Grain Sample Ticket;" or FGIS-921, "Inspection Log," to record laboratory results.

Any sample sent to TSD (including the Board of Appeals and Review) for aflatoxin testing or monitoring purposes must include the necessary information to facilitate sample processing and testing.

### 3.4 SAMPLE PORTIONS

a. Subportions.

Grind the entire sample obtained for aflatoxin testing and prepare two 500-gram subportions from the ground sample: A 500-gram work portion for original testing services and a 500-gram file sample portion for review testing. For submitted samples, retain as large a sample as possible.

For subplot testing of corn at export locations, save an additional 500-gram file (three 500-gram subportions total) for Food and Drug Administration (FDA) analysis.

From the 500-gram work portion, divide (using a Boerner divider) out a portion of 50 grams for aflatoxin testing and weigh on an FGIS-approved type scale with a minimum division size of 0.1 gram.

b. Saving File Samples.

Maintain file samples (including the FDA file sample when applicable) for all lots/samples that:

do not meet the contractual specification of the applicant for service;

are required for the aflatoxin monitoring program; or

exceed FDA action limits of 20 ppb.

When applicable, maintain a representative file sample for each lot, subplot, composite, or submitted sample tested. For submitted samples that are less than 500 grams, retain as large a sample as possible. For information concerning file sample retention periods refer to FGIS Directive 9170.13, "Uniform File Sample Retention System".

c. Storing File Samples.

If file samples are required, store each sample in a manner that will maintain the representativeness of the sample and prevent possible manipulation or substitution. Place the sample in paper bags or envelopes and label each file sample with the test date and identification. Take precautions to ensure that file sample containers are strong enough to prevent loss of sample integrity when storing samples. Do not store samples near heat, windows, or in direct sunlight. (Store samples in cold storage if available.)

d. Disposition of File Samples.

At the end of the retention period, label the file samples as follows: "FOR LABORATORY USE ONLY - NOT FOR USE AS FOOD OR FEEDSTUFF," and discard the file samples in a dumpster or landfill disposal site.

e. Shipping Samples.

When it is necessary to send samples to other laboratory locations, take precautions to maintain sample integrity by securely packaging the samples. Label the shipping container "NOT FOR HUMAN CONSUMPTION".

### 3.5 OPERATION OF GRINDERS

Samples must be ground to a fine particle size that is sufficiently fine enough to obtain a homogeneous blend. Avoid over-grinding or pulverizing a sample because it produces an excessively powdery mix that will slow down the filtration process.

Grinding must be performed in an area separate from the testing area. Use the Romer Mill - Model 2A, Bunn Grinder, or equivalent to grind the sample.

FGIS employees must follow the manufacturer's safety procedures for operating the grinder and must wear protective equipment (i.e., labcoat, mask, gloves, and hairnet) when grinding samples.

a. Romer Mill.

(1) General Operating Instructions.

The Romer Mill simultaneously grinds and subsamples corn at the rate of approximately 1 pound per minute. An adjustable restrictor door located above the collection chute varies the amount of ground sample allowed into the collection chute. Official personnel must adjust the grinder to obtain the required testing and file portions from the sample.

Adjust the grinder by locating the first line (far left) etched on the restrictor door. Position the door approximately 1/3 of the way between the first and second line. For a 10-pound sample, approximately 500 grams will be collected through the collection chute.

Once the grinder is adjusted to obtain the 500-gram sample, mark the location of the setting. To increase the sample size, move the restrictor door to the left.

If a composite sample is required in addition to the subplot-by-subplot analysis, adjust portion sizes as needed to obtain an adequate size composite and still maintain individual file samples. Obtain the composite sample from the ground subplot samples.

(2) Grinding the Sample.

Grind the entire 10-pound sample with the grind lever set at the finest range.

**NOTE: Samples with moisture content of 20 percent or more may cause the grinder motor to overheat and the breaker switch to release. If this occurs, allow the motor to cool and then set the grind lever to the coarsest setting by turning it counterclockwise. Do not grind high moisture samples on the fine grind setting.**

b. Bunn Grinder.

(1) General Operating Instructions.

The Bunn-O-Matic grinds corn at a rate of approximately 2 pounds per minute and has a holding capacity of approximately 3 to 4 pounds when fully closed. Official personnel must grind the entire sample (see section 3.2) and cut it down (using an FGIS-approved divider) to obtain the required testing and file portions from the sample.

(2) Grinding Samples.

Grind the entire 10-pound sample with the grind lever set at the fine selection. Add 3 to 4 pounds at a time into the hopper until all 10 pounds are ground. If the grinder is experiencing difficulty (e.g., over-heating, bogging down) at the fine setting, change the setting to coarse. After grinding the remainder of the sample at the coarse setting, switch the setting back to fine. Collect the entire 10-pound portion and regrind at the fine setting.

**NOTE: Samples with moisture content of 20 percent or more may cause the grinder motor to overheat and the breaker switch to release. If this occurs, allow the motor to cool and then set the grind lever to the coarse setting. Do not grind high moisture samples on the fine grind setting.**

### 3.6 CLEANING GRINDERS

A small amount of ground sample will remain in the grinder after the total sample has been ground. To prevent the contamination of subsequent samples, clean the grinder using one of the following cleaning procedures:

a. If a Vacuum Cleaner is Available.

After a sample has been ground and collected, with the unit turned on, use a vacuum cleaner with an attachment that will fit over the mouth of the chute. Place the attachment at the bottom of each chute for about 30 seconds. After all the chutes have been cleaned, turn the power off and prepare for the next sample.

b. If a Vacuum Cleaner is Not Available.

Clear the grinder by discarding a small portion (first 10 to 15 grams) of the next sample to be tested.

- (1) Pour the sample into the grinder and turn it on long enough to collect the first 10 to 15 grams.
- (2) Turn the power off, and discard the 10-15 grams ground sample.
- (3) Turn the power back on and finish grinding the sample to collect the remaining subsample for analysis.

### 3.7 CHECKING PARTICLE SIZE

a. Procedures for Checking the Performance of the Grinder.

For locations that perform mycotoxin testing on coarse (e.g., corn) and small grains, perform the check using a 100-gram sample portion of corn using the following procedures.

- (1) Grind a sample portion of approximately 100 grams of corn having a moisture content of 14.0 percent or less.
- (2) Weigh the entire portion that was ground.
- (3) Sieve the portion across a standard No. 20 wire woven sieve.
- (4) Weigh the portion that passed through the sieve.
- (5) Determine the percent of fine material, by weight, as follows:

$\text{Fines} = \text{weight from step (4) divided by the weight from step (2)} \times 100.$

b. Optimum Particle Size.

The optimum range for particles of coarse and small grain passing through the No. 20 sieve is between 60 and 75 percent. Whenever the ground particles appear to be too coarse, or the results of a grinder check indicate that less than 50 percent of the ground portion passes through the No. 20 sieve, the grinder should be adjusted or repaired to meet the optimum range requirements.

Grinding apparatuses must be checked periodically to determine whether they are producing a final product that meets the particle size requirements as listed above. Official personnel shall determine the frequency of the checks based on a number of items that include visual observation of the ground product, number of samples ground since last check, and time (number of days) since the last check was performed. Record all particle check results in a convenient location for future reference purposes.

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AFLATOXIN HANDBOOK  
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## CHAPTER 4

### CERTIFICATION

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## 4.1 BACKGROUND

Testing performed on standardized grains (e.g., corn, wheat) is performed as an official criteria factor under the authority of the United States Grain Standards Act (USGSA), as amended. Testing performed on processed grain products (e.g., corn meal) and other commodities is provided under the authority of the Agricultural Marketing Act (AMA) of 1946, as amended.

Aflatoxin results are recorded on the pan ticket, worksheet, or loading log and in the remarks section of the certificate.

Certify aflatoxin test results on grain in accordance with the USGSA/AMA (as applicable) regulations.

Upon the request of the applicant, separate certificates may be issued for grade and for aflatoxin when both are determined on the same lot.

Sections 800.125 and 800.135 of the regulations under the USGSA permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factors and official criteria may be handled separately, even though both sets of results are reported on the same certificate. When official grade or official factors and official criteria are reported on the same certificate, the review inspection certificate shall show a statement indicating that the review results are for official grade, official factors, or official criteria, and that all other results are those of the original, reinspection, or appeal inspection results, whichever is applicable.

## 4.2 GENERAL PROCEDURES

The type of service requested and the test method used determine how aflatoxin results are recorded and certified.

### a. Qualitative Testing.

- (1) Record the results of a **qualitative service** on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 20 ppb) or as exceeding the threshold.
- (2) If a **quantitative method** is used to provide qualitative service, record the test results on the work records in a quantitative measurement (e.g., 10 ppb) or a qualitative measurement (e.g.,  $\leq 20$  ppb).

(3) Certify results as being equal to or less than a threshold.

b. Quantitative Testing.

Record the results on the pan ticket and the inspection log to the nearest whole ppb.

When test results indicate that aflatoxin is present at a level of less than 5 ppb, certify the results as "Aflatoxin does not exceed 5 ppb."

Certify test results that are between 5 ppb and the conformance limit (e.g., 300 ppb) to the nearest whole ppb.

Test results greater than the conformance limit are certified as exceeding the conformance limit. For example: An aflatoxin test result of 410 ppb obtained using an aflatoxin test kit with a conformance limit of 300 ppb would result in the following certification statement: "Aflatoxin exceeds 300 ppb."

c. Certifying Test Results of Single and Combined Lots, Unit Trains, and Shiplots.

(1) Single Lot Inspection Basis for Trucks and Railcars.

Certify each test result on a separate certificate.

(2) Combined Land Carrier Basis for Trucks and Railcars.

If an applicant requests aflatoxin testing on a composite basis (up to 5 railcars and 15 trucks) and the inspection for grade on the basis of individual carriers, factor only certificates are issued for the aflatoxin testing and separate grade certificates are issued for each carrier.

(3) Composite Sample Testing for Shiplots.

Certify the composite results using the appropriate statement.

(4) Submitted Sample Testing.

Certify the results using the appropriate statement.

(5) Unit Train and Shiplot Inspection under the CuSum Loading Plan.

(a) Sublot Size for Shiplots.

The testing frequency for shiplot grain will be the same as the sample for grade analysis unless the applicant specifically requests aflatoxin analysis on the basis of a component sample.

(b) Sublot Size for Unit Trains.

The maximum size subplot for aflatoxin testing is 5 railcars for unit trains consisting of less than 200,000 bushels, or less than 50 cars. For unit trains consisting of 200,000 bushels or more, or 50 railcars or more, the maximum subplot size is 10 railcars.

For unit trains, the subplot size for aflatoxin testing and for grade analysis may be different. For example, an applicant may request grade analysis on the basis of a subplot containing two cars and request aflatoxin analysis on the basis of five cars.

(c) Recording Test Results.

Aflatoxin test results of subplot samples taken throughout loading are recorded on the loading log. A material portion occurs if the subplot result exceeds the limit as specified in the load order.

(d) Certifying Test Results.

Certify the lot based on the mathematical/weighted average (as applicable) of the accepted subplot results.

Certify material portions separately.

(e) Material Portions.

If a material portion occurs, the applicant has the option of requesting a review inspection. Review inspection results replace previous results when determining if a material portion exists.

If a material portion designation due to aflatoxin is not removed by the review inspection process, the applicant may leave the material portion on board and receive a separate certificate; return the grain to the elevator; or discharge the material portion along with additional grain in common stowage equivalent to one half the material portion quantity.

#### 4.3 STANDARD CERTIFICATION STATEMENTS

Use one of the applicable statements for certifying aflatoxin.

a. Qualitative Testing.

When aflatoxin results are equal to or less than a specific threshold (e.g., 20 ppb) ppb:

"Aflatoxin equal to or less than 20 ppb."

"Aflatoxin exceeds 20 ppb."

b. Quantitative Testing.

(1) When aflatoxin results are less than 5 ppb, use the following statement.

"Aflatoxin does not exceed 5 ppb."

(2) When aflatoxin test results are between 5 ppb and the conformance limit (e.g., 300 ppb) round to the nearest whole number in ppb.

"Aflatoxin (result rounded to the nearest whole number) ppb."

(3) When aflatoxin test results are greater than the conformance limit (e.g., 300 ppb).

"Aflatoxin exceeds (enter conformance limit) ppb."

c. Board Appeals.

Board Appeals performed by the HPLC method are certified to the nearest whole number in ppb.

"Aflatoxin (record actual results to the nearest whole number) ppb.  
Results based on High Performance Liquid Chromatography Method."

#### 4.4 OPTIONAL STATEMENTS

a. Aflatoxin Not Detected.

At the request of the applicant, use the following statement when aflatoxin is not detected (0 ppb).

"Aflatoxin not detected."

**NOTE: If subplot results are combined and averaged and the lot average is equal to 0 ppb, but an individual subplot result exceeds 0.0 ppb, the statement may not be used.**

b. Converting to Parts per Million (ppm).

At the request of the applicant, convert and certify the ppb result to parts per million (ppm) using an approved statement. To convert ppb to ppm, divide the ppb result by 1000.

"(Actual ppb result) ppb is equivalent to (converted ppm results) ppm."

c. Converting to Milligrams (mg) per Kilogram (kg), or Micrograms (µg) per Kilogram (kg).

At the request of the applicant, convert and certify results in milligrams per kilogram (mg/kg) or micrograms per kilogram (µg/kg). Use the following equivalents to determine mg/Kg or µg /kg:

$$\text{ppm} = \text{mg/kg}$$

$$\text{ppb} = \mu\text{g /kg}$$

d. Multiple Results on the Same Certificate.

When certifying multiple aflatoxin results on the same certificate and the results are based on different sample types, the certificate must reflect the difference. As a guideline, the multiple results are shown as follows:

"Sublot sample results: Aflatoxin equal to or less than 20 ppb."

"Composite sample result: Aflatoxin 14 ppb."

e. Negative Result Statement.

At the request of the applicant, one of the following statements may precede the applicable standard statements when test results are equal to or less than 20 ppb.

"The aflatoxin result is negative." OR "Negative aflatoxin."

f. Type of Test Statement.

At the request of the applicant, use this statement to indicate the type of aflatoxin test used.

"Results based on (indicate type of test used) method."

**NOTE: These certification statements may be modified as deemed necessary.**

#### **4.5 REVIEW INSPECTION STATEMENTS**

Use the appropriate statements listed below for reinspection, appeal, and Board appeal inspections.

- a. Results are reported on the same kind of certificate issued for the original service and supersede the previously issued inspection certificate.

"This certificate supersedes Certificate No. (number) dated (date)."

- b. The superseded certificate is null and void as of the date of the subsequent (reinspection/appeal/Board appeal) certificate.

"The superseded certificate has not been surrendered."

- c. When a file sample is used, enter the following statement on the reinspection/appeal/Board appeal certificate:

"Results based on file sample."

- d. When reporting more than one official result on the same certificate but at different levels of inspection, explain this condition using one of the following applicable statements:

"(Grade, factor, or official criteria) results based on (new/file) sample. All other results are those of the original inspection service."

"(Grade, factor, or official criteria) results based on the appeal inspection. All other results are those of the (original inspection/reinspection) service."

"(Grade, factor, or official criteria) results based on the Board appeal inspection. All other results are those of the (original inspection/reinspection/appeal inspection) service."



## CHAPTER 6

RESERVED



## 8.6 CLEANING LABWARE

### a. Negative Tests (# 20 ppb).

#### (1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

#### (2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

### b. Positive Tests (> 20 ppb).

#### (1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

#### (2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution." Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

## 8.7 WASTE DISPOSAL

### a. Negative Results (# 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

## **8.8 EQUIPMENT and SUPPLIES**

- a. Fluorometer - Romer model RL-100, Vicam Series III and IV, or Vicam model MF-2000.
- b. Fluorometer calibration standards. (Vicom # 33030)
- c. Cuvette Rack. (Vicom # 21010)
- d. Pump assembly stand, double. (Vicom # 21030)
- e. Syringe, glass 10 ml. (Vicom # 34010)
- f. Syringe hand pump with coupling. (Vicom #36030)
- g. Automatic pipettor (1 ml capacity for methanol). (Vicom #20501)
- h. Automatic pipettor (1 ml capacity for developer). (Vicom #20600)
- i. Graduated cylinders - 25 ml, 100 ml, and 250 ml capacity.
- j. Aflatest-P columns. (Vicom # 12022)
- k. Cuvettes, disposable 12 x 75 mm borosilicate glass tube. (Vicom # 34000)
- l. Disposable beakers. (Vicom # 36010)
- m. Glass microfibre filter paper -Whatman 934-AH. (Vicom # 31955)
- n. Small plastic funnels.

b. Positive Tests (> 20 ppb).(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution." Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

**10.8 WASTE DISPOSAL**a. Negative Results (# 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

## 10.9 EQUIPMENT AND SUPPLIES

### a. Materials Supplied in Test Kits.

- (1) 48 antibody-coated wells.
- (2) 48 red-marked mixing wells.
- (3) 1 yellow-labeled bottle of 1.5 ml 20 ppb aflatoxin control.
- (4) 1 blue-labeled bottle of 7 ml aflatoxin-HRP conjugate solution.
- (5) 1 green-labeled bottle of 24 ml K-blue substrate solution.
- (6) 1 red-labeled bottle of 32ml red stop solution.

### b. Materials Required but not Provided.

- (1) Methanol - ACS grade or better.
- (2) Deionized or distilled water.
- (3) 250 ml graduated cylinder.
- (4) Whatman 2V folded or S&S 24 cm pleated (or equivalent) filter paper.
- (5) Filter funnel.
- (6) Sample collection tubes.
- (7) Blender with mixing jars.
- (8) Balance.
- (9) Sample grinder.
- (10) Bio Tek EL 301 Microwell strip reader with 650 nm filter.
- (11) 12-channel pipettor.

- (6) 1 vial containing 8 ml of stop solution.
- (7) 1 vial containing 25ml of 20X wash concentrate.
- (8) 4 multi-channel pipette reservoirs.

b. Materials Required but not Provided:

- (1) Methanol - ACS grade or better.
- (2) Deionized or distilled water.
- (3) 100 ml graduated cylinder.
- (4) Whatman #1 filter paper or equivalent.
- (5) Glassware with 125 ml capacity for sample extraction.
- (6) Filter funnel.
- (7) 50  $\mu$ l pipette with disposable tips.
- (8) 50 -200  $\mu$ l multi-channel pipette.
- (9) 500 ml plastic squeeze bottle.
- (10) Blender with mixing jars.
- (11) Balance.
- (12) Sample grinder.
- (13) Hyperion MicroReader™ 3 Model 4027-002 with 650 nm filter.
- (14) Timer.
- (15) Waterproof marker.
- (16) Microwell holder.

## **11.10 STORAGE CONDITIONS**

- a. Store test kits between 36° - 46° F when not in use. Avoid prolonged storage of kits at room temperature. Do not freeze test kits.
- b. Do not use reagents from other SDI aflatoxin kits with different lot numbers.
- c. Bring kits up to room temperature 64° - 86° F prior to use.
- d. Do not use kit components beyond their expiration date.